

APPARENT SELECTIVE LIBERATION OF BUTYRIC ACID FROM MILK FAT BY THE ACTION OF VARIOUS LIPASE SYSTEMS¹

Wilcox *et al.* (2) have shown recently that cell-free microbial lipases differ in their ability to release volatile fatty acids from milk fat. Evidence of apparent selective hydrolysis of butyric acid from milk fat by various non-microbial lipase systems is presented in this report.

Pancreatic lipase (General Biochemical Company), milk lipase (freeze-dried raw milk powder), and glandular lipases (prepared by Dairyland Food Laboratories) from different animal sources were investigated. Cream homogenized at 2,500 p.s.i. was used as the substrate. The enzyme systems were prepared to 0.2 M pH 6.6 phosphate buffer. The concentration of enzyme was adjusted so that the reaction rate was linear in respect to enzyme concentration. The enzyme preparation and substrate were combined so that the fat content of the reaction mixture was 10%. The samples were reacted with gentle shaking at 37° C. for 5 hours. The total free fatty acids and free butyric acids were measured after 1, 3, and 5 hours of incubation (1). The results were calculated as the per cent butyric acid present in the total free fatty acid mixture (based on a microequivalent basis).

The relative per cent butyric acid varied be-

lyzed from milk fat. Although butyric acid is present in about 10 M % in unhydrolyzed milk fat, several preparations (the glandular lipases) preferentially hydrolyzed butyric acid from the fat so that it was four times this concentration in the free acid fraction. Milk lipase showed slight selectivity based on the higher concentration of this acid in the free acid position, but pancreatic lipase exhibited the opposite effect.

The importance of lipase source in selecting a system for the selective liberation of butyric acid, such as is desired in the manufacture of Romano cheese, is readily apparent. Also, the selective liberation of butyric acid by some lipase systems necessitates care in the selection of assay methods. Methods for measuring total lipase activity in these instances should quantitatively measure butyric acid.

Although the differences in the relative proportion of butyric acid in samples which have undergone lipolysis may reflect selective hydrolysis, this term has been qualified by the prefix "apparent." This is necessary since the utilization of butyric acid by other enzyme systems present in the crude preparations could change the relative proportion of butyric acid. In such cases "selective" utilization would be

TABLE 1
Lipase activity and relative butyric acid content resulting from the action of various lipase preparations on milk fat

	Increase in acid degree	(% butyric acid)		
		Range	Average*	% deviation
Pancreatic lipase	16.0	8-9.5	8.7	± 3.0
Freeze-dried raw milk	4.0	12-19	14.7	± 15.0
Glandular calf lipase	12.0	32-38	34.5	± 8.3
Glandular kid lipase	18.0	38-45	42.1	± 6.1
Glandular lamb lipase	15.0	42-52	47.6	± 7.5

* Average of ten trials.

tween 1 and 3 hours but was the same at 3 hours as at 5 hours of reaction.

The data in Table 1 show the acid degree increase resulting from lipolysis and the per cent butyric acid present in the total free acids from five different enzyme preparations. The results are based on ten separate trials and are presented as the average value, range, and standard deviation from the mean.

The data presented in Table 1 for five different enzyme systems reveal marked differences in the relative concentration of butyric acid hydro-

lyzed. Further work is in progress in this regard and also to determine "apparent" selective hydrolysis of other fatty acids and the factors involved.

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REFERENCES

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- (2) WILCOX, J. C., NELSON, W. O., AND WOOD, W. A. The Selective Release of Volatile Acids from Butterfat by Microbial Lipases. *J. Dairy Sci.*, 38: 775. 1955.

¹ Technical Note 8-54, Department of Dairy Technology, Ohio Agr. Expt. Station. Supported in part by the Research and Marketing Act of 1946 through the Dairy Products Section, W.U.R.B., A.R.S., USDA, Washington, D. C.

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